# Spliceosome discards intermediates via the DEAH box ATPase Prp43p

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**To promote fidelity in nuclear pre-mRNA splicing, the spliceosome rejects and discards suboptimal substrates that have engaged the spliceosome. Whereas DExD/H box ATPases have been implicated in rejecting suboptimal substrates, the mechanism for discarding suboptimal substrates has remained obscure. Corroborating evidence that suboptimal, mutated lariat intermediates can be exported to the cytoplasm for turnover, we have found that the ribosome can translate mutated lariat intermediates. By glycerol gradient analysis, we have found that the spliceosome can dissociate mutated lariat intermediates in vivo in a manner that requires the DEAH box ATPase Prp43p. Through an in vitro assay, we demonstrate that Prp43p promotes the discard of suboptimal and optimal 5′ exon and lariat intermediates indiscriminately. Finally, we demonstrate a requirement for Prp43p in repressing splicing at a cryptic splice site. We propose a model for the fidelity of exon ligation in which the DEAH box ATPase Prp22p slows the flow of suboptimal intermediates through exon ligation and Prp43p generally promotes discard of intermediates, thereby establishing a pathway for turnover of stalled intermediates. Because Prp43p also promotes spliceosome disassembly after exon ligation, this work establishes a parallel between the discard of suboptimal intermediates and the dissociation of a genuine excised intron product.**

RNA helicase | RNA processing | small nuclear RNA | ribonucleoprotein particle | *Saccharomyces cerevisiae*

In nuclear pre-mRNA splicing, the excision of introns is catalyzed by the spliceosome, a ribonucleoprotein machine comprising five snRNAs and ∼80 conserved proteins (for reviews, see ref. 1 and references therein). This machine assembles de novo on each pre-mRNA substrate and must rearrange its components throughout the splicing cycle through the activity of at least eight DExD/H box ATPases (2). The protein and RNA components of the spliceosome recognize the conserved elements of the intron at the 5′ splice site, the branch site, and the 3′ splice site. The RNA and possibly protein components also play key roles in catalyzing splicing, which occurs by two transesterification reactions (1). In the first reaction, the branch site adenosine attacks the 5′ splice site, generating a free 5′ exon and a lariat intermediate. In the second reaction, the 5′ exon attacks the 3′ splice site, excising the intron and ligating the exons. To establish specificity in splicing, the spliceosome discriminates optimal from suboptimal substrates.

The specific pathway that discriminates against a suboptimal substrate depends on the extent to which a substrate is suboptimal. A grossly suboptimal substrate will fail to bind the spliceosome. Although such pre-mRNAs can be degraded in the nucleus (3), they can also be exported and then degraded in the cytoplasm (4–8). In contrast, optimal substrates are specifically retained in the nucleus to favor splicing (9).

A nearly optimal substrate engages the spliceosome, necessitating more sophisticated fidelity mechanisms that involve rejecting and discarding the substrate. For example, a substrate having a point mutation in an intronic consensus sequence can engage the spliceosome but fails to splice. Such substrates are discriminated against, in part, by at least three of the DExD/H box ATPases required to splice an optimal substrate (10–12). Specifically, Prp5p, which promotes binding of U2 to a substrate (13), discriminates against mutated branch site sequences (12). Prp16p, which promotes rearrangements required for exon ligation (14), discriminates against mutated branch site sequences at a later stage than Prp5p (10). Finally, Prp22p, which promotes release of the mRNA after exon ligation (15, 16), discriminates against mutated consensus sites before exon ligation (11). Fidelity is also promoted by the sequestration of suboptimal substrates through equilibration between distinct spliceosomal states (17–19).

Prp22p, if not also Prp5p and Prp16p, is insufficient to discard a nearly optimal substrate (11). The equilibration of distinct spliceosomal states is also insufficient to discard a nearly optimal substrate and to preclude splicing. Thus, additional mechanisms must contribute to fidelity and account for the discard of nearly optimal substrates. Such substrates are degraded by either nuclear or predominantly cytoplasmic exonucleases, following debranching by Dbr1p in the case of the lariat intermediate (3, 6). Whereas nuclear turnover may simply compete with splicing, cytoplasmic turnover implies that the spliceosome can dissociate a suboptimal substrate. After splicing, spliceosome disassembly and the dissociation of an optimal, excised intron require Ntr1p/Spp382p, Ntr2p, and the DEAH box ATPase Prp43p (20–25), which also functions in the processing of pre-rRNA and histone pre-mRNA (26–29). Interestingly, mutations in *PRP43* and *NTR1*/*SPP382* suppress mutations in the spliceosome assembly factors *PRP38* as well as *PRP8*, and Ntr1p/Spp382p associates in vitro with stalled spliceosomes that retain the lariat intermediate but lack the 5′ exon, hinting that the spliceosome discards intermediates by a mechanism that parallels the mechanism for discarding an optimal, excised intron product (30). Indeed, we have found in *Saccharomyces cerevisiae* that the spliceosome can utilize the intron release factor Prp43p to dissociate suboptimal substrates and to promote fidelity.

## Results

**A Suboptimal Lariat Intermediate Can Undergo Translation in the Cytoplasm.** To investigate the mechanism by which the spliceosome discards suboptimal substrates, we developed an in vivo reporter for discard in *S. cerevisiae*. Given the implied cytoplasmic localization of discarded substrates (6), we set out to design an assay

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that would reveal the discard of either pre-mRNA or lariat intermediate into the cytoplasm. To this end, we engineered a reporter that would permit translation of discarded species. This reporter includes the intron and flanking exonic sequences of *ACT1* fused downstream to *lacZ*. To permit translation of *lacZ* independent of the cap or splicing, we inserted an internal ribosome entry site (IRES) from the cricket paralysis virus (31) in the 3′ exon upstream of *lacZ* (Fig. 1*A*).

The IRES construct, which did not perturb RNA processing (Fig. 1*B*; *SI Text*, *SI Note 1*), did report on discard of suboptimal splicing species into the cytoplasm. For comparison, a wild-type intron reporter, which produces predominantly mRNA (Fig. 1*B*), yielded β-galactosidase activity 14-fold above a control reporter having a mutated, nonfunctional IRES (Fig. 1*C*; ref. 31). In contrast, in a wild-type *DBR1* strain the UAc mutated 3′ splice site reporter yielded no significant β-galactosidase activity above the control (Fig. 1*C*, lanes 5 and 6), consistent with a defect in mRNA formation and efficient turnover of the suboptimal lariat intermediate (6). However, in a *dbr1Δ* strain the stabilized discarded UAc intermediate was translated, enabling β-galactosidase activity 9-fold above the control (Fig. 1*C*, lanes 7 and 8). This finding demonstrates the previously implicated discard of stalled, lariat intermediates to the cytoplasm (6). Whereas these results do not directly address the compartment of turnover for intermediates that are readily debranched, published data indicate that debranching does not preclude localization of a discarded lariat intermediate to the cytoplasm (6).

**A Wide Range of Suboptimal Splicing Substrates Localize to the Cytoplasm.** Next, we determined the specificity for the discard pathway. In particular, we tested substrates having mutations at the first position of the intron (G1) or at the branch site (br) adenosine. These substrates are defective in 5′ splice site cleavage, exon ligation, or both and accumulate each splicing species to varying degrees (Fig. 2, *Top* and *Middle*; e.g., ref. 32). Specifically, by primer extension analysis the G1a and brG substrates formed little mRNA and accumulated predominantly lariat intermediate. Nevertheless, β-galactosidase assays revealed that the G1a and brG substrates expressed *lacZ* at levels 22- and 40-fold above the control, the nonexpressing UAc substrate in a wild-type *DBR1* strain (Fig. 2, *Bottom*; cf. bars 2 and 5 with 8). This high level of expression is greater than that for the wild-type substrate and correlates well with the increased levels of 3′ exon (Fig. 2*B*)—as reflected in the strong accumulation of the lariat intermediates, whose mutated branch structures are resistant to Dbr1p (33). Given the predominantly cytoplasmic localization of wild-type mRNA, these data provide evidence that the G1a and brG lariat intermediates do not stall on the spliceosome in the nucleus but rather localize to and accumulate in the cytoplasm.

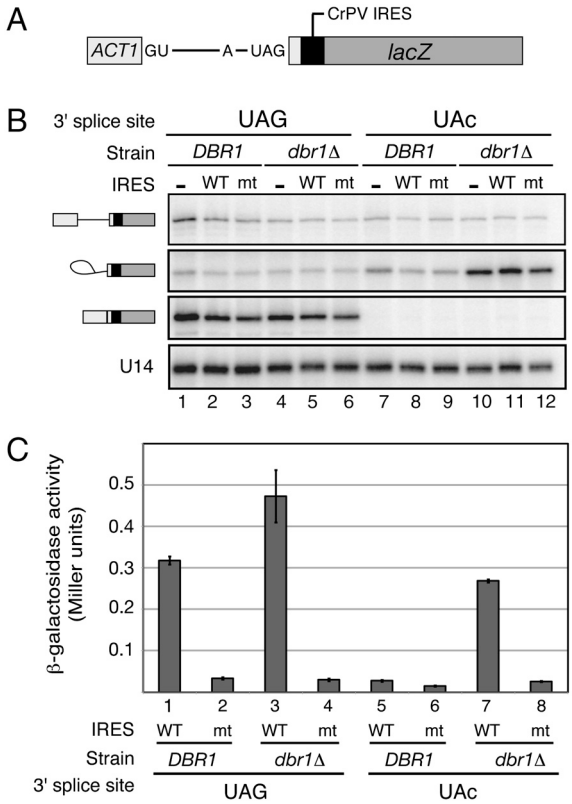


**Fig. 1.** Translation of a suboptimal splicing intermediate. (*A*) The *ACT1-IRES-lacZ* discard reporter. (*B* and *C*) Reporters in *DBR1* (H2545) or *dbr1Δ* (yJPS1080) were analyzed for RNA by extending a 3′ exon primer (*B*) or for protein by β-galactosidase activity (*C*). (*B*) Splicing and discard are unchanged by the wild-type (WT) or mutated (mt) IRES. U14 is an internal control. (*C*) IRES-dependent translation of a discarded lariat intermediate. Error bars reflect the range for two experiments.

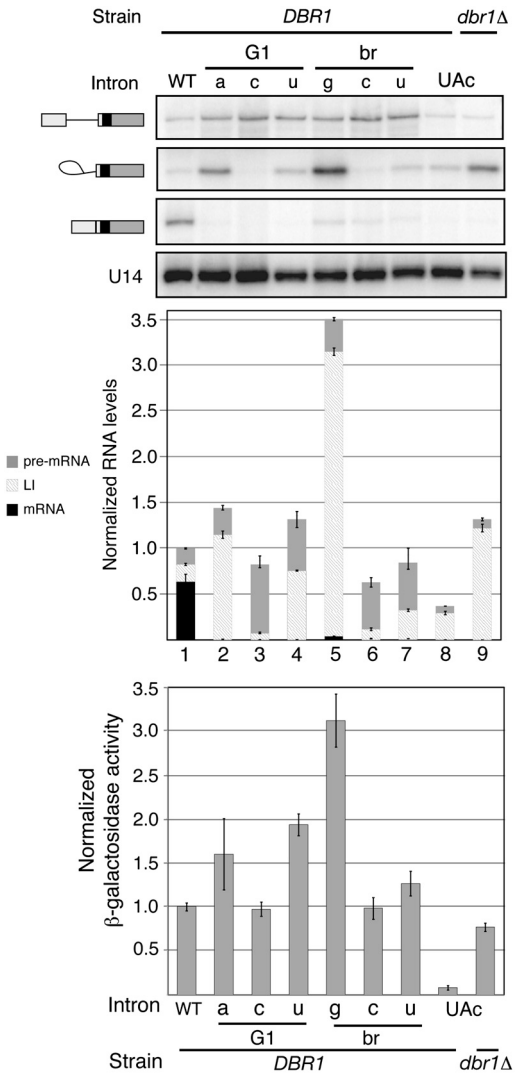


**Fig. 2.** A wide range of suboptimal substrates is discarded into the cytoplasm. Discard reporters, in *DBR1* (H2545) or *dbr1Δ* (yJPS1080), were wild type (WT) or mutated at the 5′ splice site (G1), branch site (br), or 3′ splice site. (*Top*) RNA was analyzed as in Fig. 1*B*. (*Middle*) Levels of pre-mRNA (*Light Gray*), lariat intermediate (*Diagonal Stripes*), and mRNA (*Black*) were stacked and normalized to wild-type levels to reflect total 3′ exon levels. (*Bottom*) β-galactosidase activity, relative to wild type. Activity correlates with total 3′ exon levels. Error bars reflect the range for two experiments.

The G1c and brC substrates formed little mRNA and accumulated predominantly pre-mRNA (Fig. 2, *Top* and *Middle*). Nonetheless, these suboptimal substrates expressed *lacZ* approximately 12-fold above the control, similar to the wild-type substrate (Fig. 2, *Bottom*; cf. bars 1, 3, and 6). These similar levels of expression correlated with the similar levels of suboptimal pre-mRNAs and optimal mRNA (Fig. 2, *Middle*; cf. lane 1 black bar with lanes 3 and 6 solid gray bars). These data indicate that, as for optimal mRNA, the suboptimal pre-mRNAs also accumulate in the cytoplasm, consistent with previous observations (e.g., refs. 4 and 6). Finally, the G1u and brU substrates formed little mRNA and accumulated similar levels of pre-mRNA and lariat intermediate that together were comparable to wild-type mRNA levels (Fig. 2, *Top* and *Middle*; cf. lanes 1, 4, and 7). These substrates also expressed *lacZ* as efficiently as the wild-type substrate (Fig. 2, *Bottom*). Thus, for all substrates, the expression of β-galactosidase correlated well with the total primer extension signal from all IRES-containing splicing species (Fig. 2; cf. *Middle* and *Bottom*; Fig. S1). Indeed, the ratios of β-galactosidase to 3′ exon for the mutated reporters were, on average, within 30% of the ratio for the wild-type reporter, despite widely varying splicing efficiencies. These data provide evidence that substrates having mutations at any of the intronic consensus sequences can not only discard to the cytoplasm but also accumulate predominantly in the cytoplasm, regardless of whether the substrate compromises 5′ splice site cleavage, exon ligation, or both (*SI Text*, *SI Note 2*).

**Suboptimal Intermediates Dissociate from the Spliceosome.** To test the implication that discarded intermediates were dissociated from the spliceosome, we lysed yeast expressing various *ACT1-CUP1* splicing reporters (32), lacking an IRES element, and then determined the migration of splicing species on a glycerol gradient. In a wild-type *DBR1* strain expressing a mutated UAc 3′ splice site reporter, a significant population of the lariat intermediate and 5′ exon migrated rapidly, peaking in fractions 24–26 (Fig. 3*A*), indicating that these intermediates remained bound to the spliceosome (*SI Text*, *SI Note 3*). Nonetheless, a second population of lariat intermediate migrated slowly, peaking in fractions 10–14 (Fig. 3*A*, first panel), suggesting that these lariat intermediates were discarded. Indeed, in a *dbr1Δ* strain only the slowly migrating population of lariat intermediate increased, relative to the *DBR1* strain (Fig. 3*A*, third panel; Fig. S2*C*). In contrast, the slowly migrating population of 5′ exon did not increase (Fig. 3*A*; *SI Text*, *SI Note 4*). The slowly migrating pool of Dbr1p-sensitive lariat intermediate correlates with the cytoplasmic pool of discarded lariat intermediate, inferred from the IRES reporter (Fig. 2; ref. 6). In a strain deleted for *SKI2*, a cytoplasmic cofactor for the exosome nuclease complex, the UAc lariat intermediate was stabilized in the slowly migrating fractions by 3.9-fold, providing independent evidence for cytoplasmic localization of this species (Fig. 3*B*). Moreover, in the *ski2Δ* strain, a slowly migrating population of 5′ exon was stabilized by 1.8-fold, thereby providing evidence that the spliceosome also discards a 5′ exon by dissociation that similarly leads to localization and turnover in cytoplasm (Fig. 3*B*).

The spliceosome also discarded substrates having mutations at the branch site or the 5′ splice site by dissociating the corresponding lariat intermediates (*SI Text*, *SI Note 5*). Thus, as suggested by the IRES reporters (Fig. 2), the spliceosome can dissociate a broad range of intermediates—whether the substrate is mutated at the 5′ splice site, the branch site, or the 3′ splice site.

**Dissociating Intermediates from the Spliceosome Requires Prp43p.** To test the hypothesis that factors required for release of a genuine intron product are also required for discard of a suboptimal intermediate, we investigated the role of the DEAH box ATPase Prp43p in discard. Specifically, we asked whether the cold-sensitive *prp43-Q423N* mutant (28) was impaired for dissociation

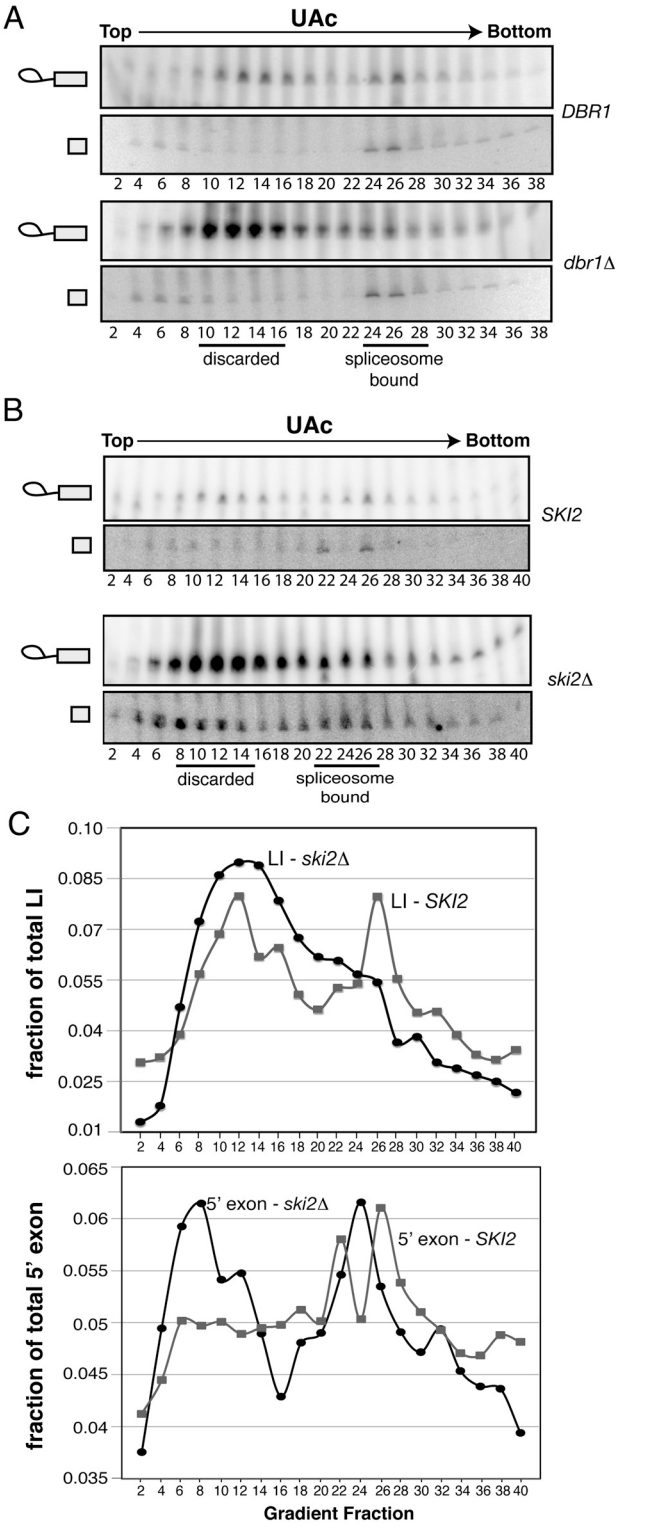


**Fig. 3.** In vivo, both intermediates of a suboptimal substrate are dissociated from the spliceosome and degraded in the cytoplasm. Lysates of cells expressing the mutated UAc 3′ splice site *ACT1-CUP1* splicing reporter were fractionated by glycerol gradient and then assayed for lariat intermediate as in Fig. 1*B* or for 5′ exon by northern. (*A*) A discarded lariat intermediate is dissociated from the spliceosome. The reporter was analyzed in a wild-type *DBR1* (BY4741) or mutant *dbr1Δ* (yJPS799) strain. Data in bottom panels are quantitated in Fig. S2*C*. (*B*) A discarded 5′ exon is dissociated from the spliceosome and degradation of both discarded intermediates requires a cofactor of the cytoplasmic exosome. The reporter was analyzed in a wild-type *SKI2* (BY4741) or mutant *ski2Δ* (yJPS979) strain. (*C*) Quantitation of the lariat intermediate (LI; *Top*) or 5′ exon (*Bottom*) levels in *B*, normalized to the total levels of each species across the gradient.

of stalled intermediates from the spliceosome. Specifically, we transformed the double mutant strain *prp43-Q423N dbr1Δ* with a 3′ splice site mutated *ACT1-CUP1* splicing reporter (32), shifted cells to the restrictive temperature, lysed the cells, and analyzed the migration of splicing species on a glycerol gradient. As expected, with a wild-type substrate the excised lariat intron migrated slowly in the wild-type *PRP43* strain but rapidly in the *prp43-Q423N* mutant strain (Fig. S2*E*; refs. 23 and 24). Strikingly, the proportion of UAc lariat intermediate that migrated rapidly with the spliceosome doubled from only 34% in the wild-type *PRP43* strain to 65% in the *prp43-Q423N* mutant strain (Fig. 4), suggesting that the spliceosome requires Prp43p to dissociate suboptimal lariat intermediates.

To test for a direct requirement for Prp43p in discard, we first established an in vitro assay for the dissociation of suboptimal lariat intermediates from the spliceosome utilizing a *UBC4* pre-mRNA that (*i*) has a short intron that splices efficiently in vitro, as found by John Abelson (see also ref. 34), and (*ii*) is, like *ACT1* (11), proofread by Prp22p at the exon ligation stage (Fig. S3*A*). A *UBC4* pre-mRNA having a UgG 3′ splice site mutation accumulated lariat intermediate and decreased splicing at the mutated site but stimulated splicing at a cryptic splice site six nucleotides upstream of the mutated splice (Fig. 5*A*). Importantly, in a *dbr1Δ* extract the stalled UgG lariat intermediate was stabilized and migrated on a glycerol gradient as slowly as the released, wild-type excised intron, indicating that the spliceosome dissociated the mutated intermediate (Fig. 5 *E* and *F*; cf. first and third panels, peak fraction 4; Fig. S4*B*).

In *dbr1Δ* extract the slowly migrating, wild-type lariat intermediate was also stabilized (Fig. 5*E*; cf. first and third panels, peak fraction 4; Fig. S4*A*), suggesting that a proportion of the wild-type intermediate is also discarded, as inferred in vivo (6). Unexpectedly, the proportion of lariat intermediate that was discarded for the wild-type substrate (37%) was as high as for the UgG substrate (34%) (Fig. 5 *G* and *H*; Fig. S4 *C* and *D*), suggesting that the discard pathway does not inherently distinguish between optimal and suboptimal substrates. Note, however, that a greater amount of mutated lariat intermediate was discarded given the stronger accumulation of this intermediate (30%) compared to the wild-type intermediate (8%), relative to total substrate levels. In contrast to the UgG lariat intermediate, we did not observe a slowly migrating population of the cognate 5′ exon in the *dbr1Δ* extract (Fig. 5*J*, third panel, and Fig. S4*F*, first panel; *SI Text*, *SI Note 4*). Thus, the spliceosome not only discards intermediates in vivo but also in vitro.

We next tested whether Prp43p was required for discard of the mutated *UBC4* lariat intermediate by supplementing extract with rPrp43p having the dominant negative mutation Q423E (*SI Text*, *SI Note 6*). As expected (23), in *dbr1Δ* extract rPrp43p-Q423E shifted the wild-type excised intron from slowly migrating fractions to rapidly migrating, spliceosome-containing fractions (Fig. 5*E*; cf. third and fourth panels, peak fraction 14; Fig. S4*A*). Importantly, for both the wild-type and UgG 3′ splice site substrates in *dbr1Δ* extract, rPrp43p-Q423E also shifted the discarded lariat intermediates from the slowly migrating fractions to the rapidly migrating fractions (Fig. 5 *E* and *F*; cf. third and fourth panels; Fig. S4 *A* and *B*; *SI Text*, *SI Note 7*). Further, rPrp43p-Q423E shifted the optimal lariat intermediate as efficiently as the suboptimal intermediate (Fig. 5 *G* and *H* and Fig. S4 *C* and *D*), suggesting that Prp43p does not distinguish suboptimal from optimal substrates. Thus, Prp43p is required to dissociate lariat intermediates from the spliceosome not only in vivo but also in vitro.

We found that Prp43p is also required to discard the 5′ exon. For both the wild-type and UgG substrates, rPrp43p-Q423E increased the levels of 5′ exon both in splicing reactions and in the spliceosome-containing fractions (Fig. 5 *I* and *J*; cf. first with second and third with fourth panels; Fig. S4 *E* and *F*; *SI Text*, *SI Note 8*). Further, Prp43p-Q423E increased the levels of the 5′ exon from the wild-type substrate as efficiently as for the UgG substrate (Fig. 5 *K* and *L*), suggesting again that the discard pathway does not inherently distinguish between optimal and suboptimal substrates. Together with our in vivo data (Fig. 3), these findings imply that Prp43p dissociates both the lariat intermediate and cognate 5′ exon (*SI Text*, *SI Note 9*). In contrast, though Prp22p represses exon ligation at a suboptimal 3′ splice site, Prp22p is not required for discard of splicing intermediates (Fig. S3 *B–E*; *SI Text*, *SI Note 10*).

Consistent with a role for Prp43p in dissociating both the 5′ exon and lariat intermediate, we discovered a role for Prp43p in the fidelity of exon ligation. Specifically, mutated rPrp43p-Q423E increased not only the levels of intermediates from a UgG 3′ splice site substrate but also the levels of the cryptic mRNA product (Fig. 5*A*, lane 6; Fig. 5 *B–D*). In contrast, mutated rPrp43p-Q423E did not significantly increase the levels of mRNA from a wild-type substrate. Thus, Prp43p not only discards intermediates but also represses formation of a cryptic mRNA, thereby enhancing the specificity of exon ligation.

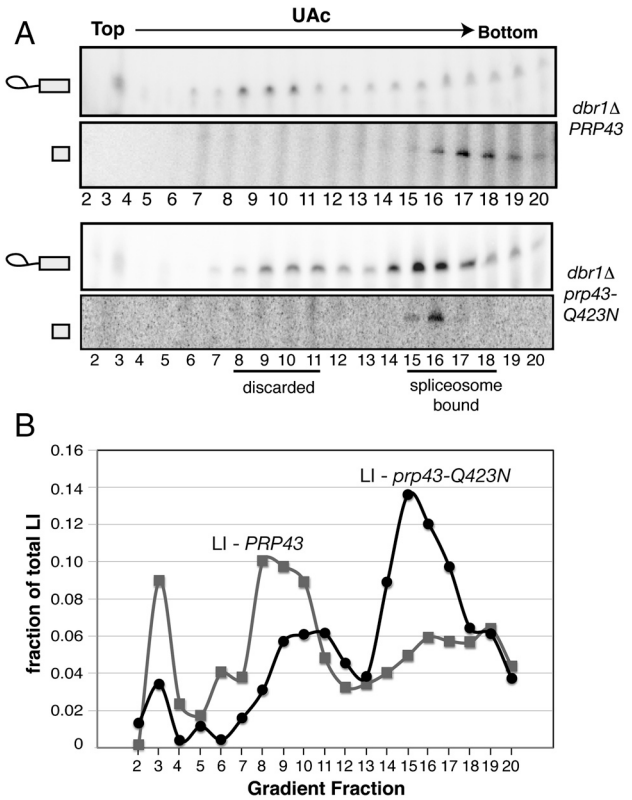


**Fig. 4.** In vivo, the discard of a suboptimal intermediate from the spliceosome requires the DEAH box ATPase Prp43p. (*A*) The discarded UAc lariat intermediate remains spliceosome-bound in the *prp43-Q423N* mutant. The mutated 3′ splice site reporter was investigated in a wild-type *PRP43* or mutant *prp43-Q423N* strain each deleted for *DBR1*. Lysates were analyzed as in Fig. 3. (*B*) Quantitation of the levels of lariat intermediate in *A*, normalized to the total levels across the gradient.

## Discussion

Suboptimal lariat intermediates can be turned over by predominantly cytoplasmic nucleases (6), suggesting that the spliceosome discards such intermediates through dissociation. Using a translation-based assay, we confirmed that the spliceosome can discard suboptimal pre-mRNA and lariat intermediate into the cytoplasm—regardless of the consensus site mutated (Figs. 1 and 2). By glycerol gradient analysis, this discard involves dissociation of both intermediates of a suboptimal substrate from the spliceosome in vivo (Fig. 3), and the dissociation of the lariat intermediate, at least, requires Prp43p (Fig. 4). By establishing

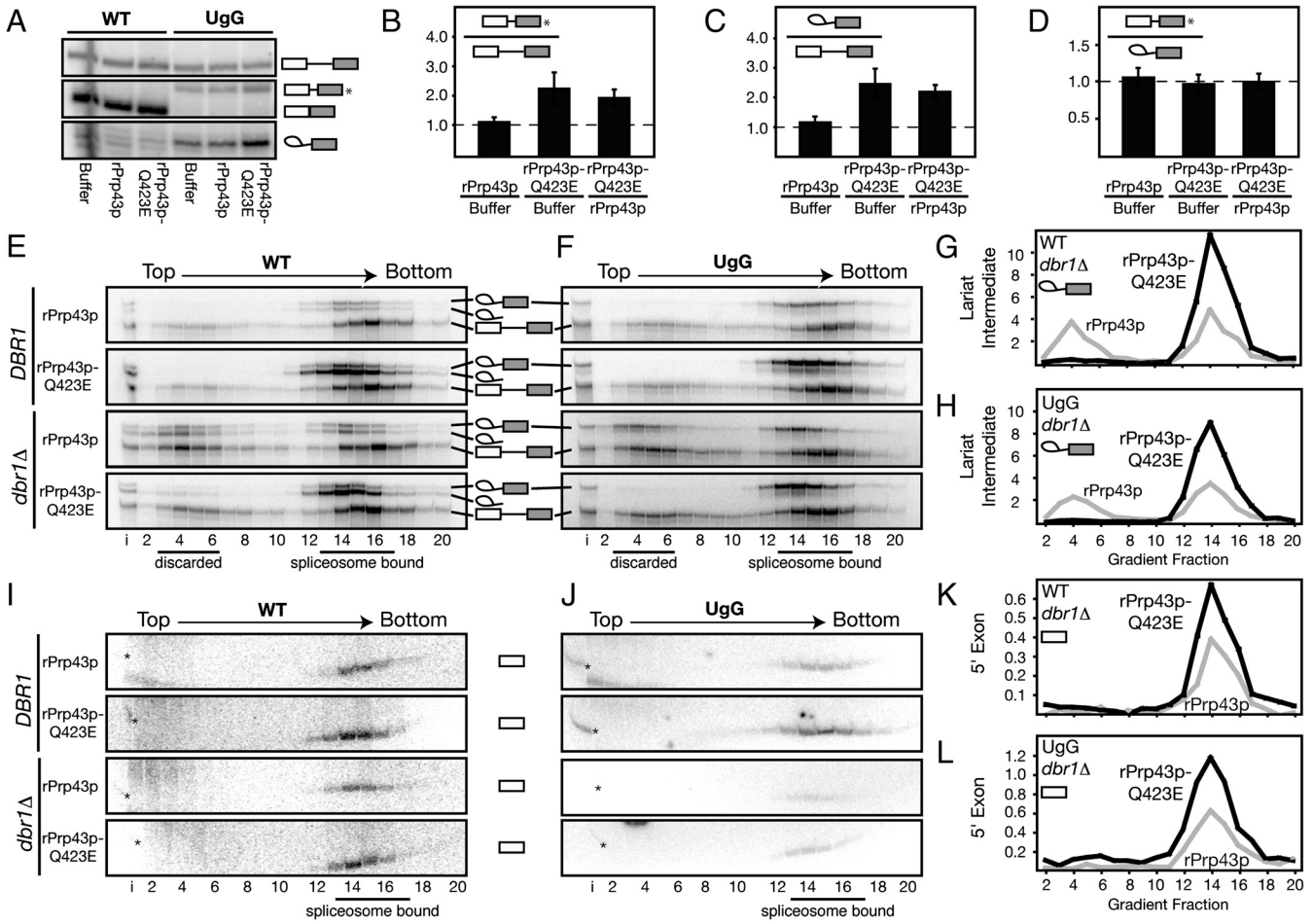


**Fig. 5.** Prp43p is required in vitro to discard the lariat intermediate and 5′ exon from the spliceosome and to minimize formation of a cryptic mRNA. (*A*) Unlabeled *UBC4* pre-mRNA having a wild-type (WT) or mutated UgG 3′ splice site was spliced in wild-type extract with buffer, rPrp43p, or rPrp43p-Q423E and analyzed by extension of a 3′ exon primer. The asterisk marks the cryptic mRNA product. (*B–D*) Prp43p represses splicing at a cryptic 3′ splice site. The UgG data in *A* are quantitated. Error bars indicate standard deviation for five independent experiments. (*E*, *F*, *I*, and *J*) Radiolabeled *UBC4* pre-mRNA having a wild-type (*E* and *I*) or mutated UgG (*F* and *J*) 3′ splice site was spliced in either wild-type *DBR1* or mutant *dbr1Δ* extracts supplemented with either wild-type rPrp43p or mutated rPrp43p-Q423E. Splicing reactions were fractionated by glycerol gradient; the input (i) and fraction numbers are shown. Asterisks indicate the migration of the 5′ exon in the input lane. Visualization of the 5′ exon levels was optimized by adjusting the brightness and contrast in Adobe Photoshop. (*G*, *H*, *K*, and *L*) Quantitation of the lariat intermediate (*G* and *H*) or 5′ exon (*K* and *L*) for the UAG (*G* and *K*) or UgG (*H* and *L*) substrates spliced in *dbr1Δ* extract supplemented with wild-type rPrp43p (*Gray Line*) or rPrp43p-Q423E (*Black Line*). Data are from *E*, *F*, *I*, and *J* and are normalized to the input levels of lariat intermediate.

an in vitro assay for dissociation with two different substrates, we confirmed a direct role for Prp43p in discard and turnover of both the lariat intermediate and 5′ exon (Fig. 5). Furthermore, we found that Prp43p represses splicing at a cryptic 3′ splice site in *UBC4* (Fig. 5). Thus, our data establish evidence that the DEAH box ATPase Prp43p reiterates its function in dissociating optimal, excised introns to discard suboptimal intermediates and to promote fidelity in exon ligation.

Consistent with a role for Prp43p in discarding intermediates, Prp43p and its cofactors, Ntr1p/Spp382p and Ntr2p, associate with spliceosomes stalled at the exon ligation stage (35). Furthermore, Ntr1p/Spp382p associates with spliceosomes that contain the lariat intermediate but lack the 5′ exon (30). Although this latter, circumstantial observation does not distinguish whether Prp43p functions before or after discard of the 5′ exon, we show in vitro that the Prp43p-Q423E mutation compromises dissociation of both the lariat intermediate and the 5′ exon of *UBC4* (Fig. 5) and turnover of both intermediates of *ACT1* (Fig. S4 *K* and *L*). Supporting a role for Prp43p in discarding intermediates, the Cheng lab has found that a purified complex of Prp43p, Ntr1p/Spp382p, and Ntr2p can disassemble affinity-purified spliceosomes stalled at specific stages in the splicing pathway.

Our results imply a model for establishing the fidelity of exon ligation in which Prp22p and Prp43p cooperate to ensure the rejection and discard of suboptimal substrates (Fig. S5). In this model, suboptimal intermediates are preferentially stalled, because of Prp22p-mediated rejection, inefficient splicing, or both (11), and Prp43p prohibits the accumulation of spliceosomes containing such intermediates by dissociating these intermediates from the spliceosome.

At the catalytic stage of splicing, the spliceosome assumes two catalytic conformations and an intermediate conformation (17–19). Although Prp43p could conceivably discard splicing intermediates from any of these conformations, Prp43p may act inefficiently on the catalytic conformations, because Prp16p and Prp22p bind the first and second catalytic conformations, respectively (11, 14, 15, 17, 36), and Prp16p, Prp22p, and Prp43p appear to bind to the spliceosome mutually exclusively (37, 38). Instead, Prp43p may act on the intermediate conformation characterized by the stem IIa configuration of U2 snRNA, which is mutually exclusive with the stem IIc configuration characteristic of the catalytic conformations. The toggling between the stem IIa and IIc states throughout splicing (18, 39) may provide multiple entry points to Prp43p before, during, and after catalysis. It

will be important to determine whether additional disassembly factors, including the RNA helicase Brr2p and the GTPase Snu114p (25), also promote discard of suboptimal substrates.

Prp43p may discard not only suboptimal intermediates but also suboptimal pre-mRNAs (e.g., Fig. 2), which also localize to the cytoplasm (4–7). Grossly suboptimal pre-mRNAs likely fail to bind the spliceosome, but nearly optimal pre-mRNAs likely engage the spliceosome, thereby necessitating ATP-dependent rejection and Prp43p-mediated discard. Indeed, mutations in spliceosome assembly factors that accumulate pre-mRNA are suppressed by *prp43* mutations (30). Finally, the role of Prp43p in the processing of pre-rRNA and histone pre-mRNA (26–29) raises the intriguing possibility that Prp43p may mediate discard pathways in these processes as well to promote the fidelity of pre-rRNA and histone pre-mRNA processing.

Suboptimal substrates are turned over not only in the cytoplasm but also in the nucleus. However, nuclear turnover can occur without discard from the spliceosome, because defects in nuclear turnover yield increased mRNA—even from suboptimal intermediates (3). By dissociating substrates, Prp43p can contribute further to fidelity by aborting a round of splicing. A discarded pre-mRNA may subsequently export to the cytoplasm and degrade, but the pre-mRNA may also rebind the spliceosome, challenging the fidelity mechanism yet again. In contrast, a discarded intermediate is committed to turnover. However, with a synthetic IRES-dependent reporter, discarded lariat intermediates, which are effectively capped at the 5′ end, can alternatively engage the ribosome and code for a protein product (Figs. 1 and 2). Intriguingly, in *Didymium iridis* a group I-like ribozyme cleaves the I-*Dir*I transcript, forming a small spliceosome-like lariat that substitutes for the cap structure at the 5′ end of the mRNA, thereby stabilizing the message and allowing for translation (40). Thus, our work also raises the possibility that spliceosome-generated lariat intermediates may be utilized by the cell as translation substrates. Further, in *Schizosaccharomyces pombe* telomerase RNA corresponds to a 5′ exon (41), suggesting that the rejection and discard of intermediates may also be important in the biogenesis of such functional noncoding RNAs. For example, the long branch site to 3′ splice site distance in the telomerase RNA intron may impede exon ligation by rendering the substrate sensitive to Prp22p-mediated rejection and Prp43p-mediated discard, thereby liberating the 3′ processed telomerase RNA. Finally, given the established role of Ntr1p/Spp382p in the activation of Prp43p (20), additional regulatory factors could target Prp43p to effectively modulate the partitioning of a substrate between discard and productive splicing and thereby to regulate this stage of RNA processing.

## Materials and Methods

Strains (Table S1), plasmids (Table S2), and further experimental details are provided in *SI Text*.

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